

09/914036

CLAIMS

- 5
10
15
20
25
30
35
1. A method for purifying a crude viral preparation containing viral particles of interest, characterized in that it comprises at least one fluidized-bed adsorption step.
2. The method as claimed in claim 1, characterized in that said fluidized bed contains particles of adsorbent and is obtained by suspending said particles in a fluid under the action of one or more forces selected from mechanical, electromagnetic, magnetic, gravitational and electrical forces.
3. The method as claimed in claim 2, comprising:
- a) a phase for expanding said particles of adsorbent in a chromatography column, in particular by applying an ascending flow of buffer, said expansion phase being maintained until a fluidized bed is obtained,
 - b) a phase for loading said crude viral preparation, in particular in the lower part of said column,
 - c) a phase for washing by passing a buffer through, in particular in an ascending flow,
 - d) a phase for sedimentation, optionally aided by a descending flow of buffer,
 - e) a step for elution by applying a flow of buffer, in particular a descending flow, in order to allow the release of the viral particles adsorbed onto said particles of adsorbent.
4. The method as claimed in claim 2 or 3, characterized in that said particles of adsorbent consist of polymer, and more particularly of a

polymer chosen from agarose, polyacrylamide, polystyrene or derivatives thereof.

5. The method as claimed in one of the preceding claims, characterized in that said particles of adsorbent bear at least one ligand capable of binding specifically and reversibly to an antiligand, said antiligand consisting of all or part of a said viral particle of interest.

6. The method as claimed in ~~claim~~ 5, characterized in that said ligand consists of a positively charged group, advantageously a basic group, and more particularly a group selected from the dimethylaminoethyl (DMAE) group, the diethylaminoethyl (DEAE) group, the trimethylaminoethyl (TMAE) group, the group $-R-CH(OH)-CH_2-N^+-(CH_3)_3$ (Q group), the guanidinium group or the imine group, such as polyethyleneimine (PEI).

7. The method as claimed in one of ~~claims~~ 2 to 6, characterized in that said particles of adsorbent consist of an agarose matrix and comprise a central core made of quartz and dextran chains covalently coupled to said agarose matrix, on which is attached said positively charged group and, in particular, the Q group.

8. The method as claimed in any one of ~~claims~~ 1 to 7, characterized in that it is carried out under conductivity conditions of between approximately 25 and approximately 70 mS/cm, advantageously between approximately 30 and approximately 40 mS/cm, and preferably between approximately 30 and approximately 35 mS/cm.

9. A protocol for producing viral particles which can be used for gene therapy, comprising the following steps (i) and (ii):

(i) production of a crude viral preparation, comprising the steps:

(a) infecting or transfecting a suitable cell line with at least one viral vector of interest, preferably a recombinant viral vector of interest;

(b) culturing said infected or transfected cell line under conditions which allow viral replication and the production of viral particles;

(c) collecting the cells and/or the supernatant,

(ii) purification of said crude viral preparation according to one of the methods of claims 1 to 8.

10. The protocol as claimed in claim 9, characterized in that it also comprises:

(i) a cell rupture or lysis step after step (c), optionally followed by a step for degrading the nucleic acids,

(ii) a step for inactivating enveloped viruses, and/or

(iii) a packed-bed chromatography step, and in particular a gel filtration chromatography step.

11. The method as claimed in one of claims 1 to 8 or the protocol as claimed in claim 9 or 10, characterized in that said viral particles are adenoviral particles.